

Supplemental Data

Deconstructing the Cadherin-Catenin-Actin Complex

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Supplemental Experimental Procedures

Prokaryotic Expression Vectors

Full-length murine β -catenin and human plakoglobin were cloned into the pGEX-TEV vector, which results in a N-terminal TEV cleavable GST-tag. To obtain full-length $\beta\alpha$ -cat the XhoI fragment of a pGEX-TEV- α -catenin 56-906 expression vector was ligated into the XhoI cut pGEX-2T- $\beta\alpha$ -cat vector (Pokutta and Weis, 2000). The sequence of the $\beta\alpha$ -catenin full-length construct was confirmed by sequencing. The construct of COMP-E-cadherin was generated by overlap extension PCR. A fragment of rat COMP residue 27 to 83 was generated by PCR and a 4 glycine linker was introduced with the 3' primer. The 5' primer used to amplify the murine E-cadherin sequence 580-729 was designed to overlap with the 3' primer of the COMP fragment. Using these PCR products in a subsequent PCR reaction yielded the final product, which was cloned into a pGEX-TEV vector.

Protein Expression and Purification

Proteins were expressed in *E.coli* BL21 cells. GST-fusion proteins were expressed and purified as described previously (Pokutta and Weis, 2000). GST-E-cadherin and GST-COMP-E-cadherin proteins were eluted from GST-agarose with 50 mM reduced glutathione, 20 mM Tris pH 8.5, 150 mM NaCl and 1mM DTT. Further purification was done by FPLC using a Mono Q column (GE Healthcare) for GST-E-cadherin and Superdex 200 for GST-COMP-E-cadherin. GST- β -catenin was cleaved from Glutathione agarose beads with TEV (0.03 mg/ml 50% beads for 1h at room temperature) and subsequently purified by anion exchange (Mono Q, GE Healthcare) followed by size exclusion (Superdex 200, GE Healthcare) chromatography. GST- $\beta\alpha$ -catenin was cleaved from the Glutathione agarose beads with bovine thrombin (Sigma, 20U/ml 50% beads for 4h) and purified on a Mono Q anion exchange column. Plakoglobin was purified as described for β -catenin, except that the incubation time with TEV was increased to 3h at room temperature followed by overnight at 4°C, and the anion exchange step was omitted. His6-tagged α -catenin full-length was purified as described before (Pokutta and Weis, 2000) with the difference that detergent free lysis buffer was used. After purification on the Superdex 200 column monomer and dimer peak fractions of α -catenin were concentrated separately and further purified on an analytical Superdex 200 column. His6-tagged vinculin head domain was expressed in BL21 cells and purified as described for His6-tagged α -catenin.

Mammalian Expression Vectors

Canine E-cadherin-GFP (Adams et al., 1998), murine α -catenin (Herrenknecht et al., 1991) and murine β -catenin (Butz et al., 1992) were subcloned into pEGFP-N1 (cadherin) and C1 (catenin) vectors. The C-termini of E-cadherin (amino acids 756-887) and α -catenin (amino acids 671-906) were deleted by digesting two internal XmaI sites in each sequence. The E-cadherin Δ C construct was cloned into tandem-dimer DsRed, ptdDsR-N1 (Campbell et al., 2002). Photoactivatable GFP (Patterson and Lippincott-Schwartz, 2002) was fused to murine β -actin (Robbins et al., 1999).

Supplemental References

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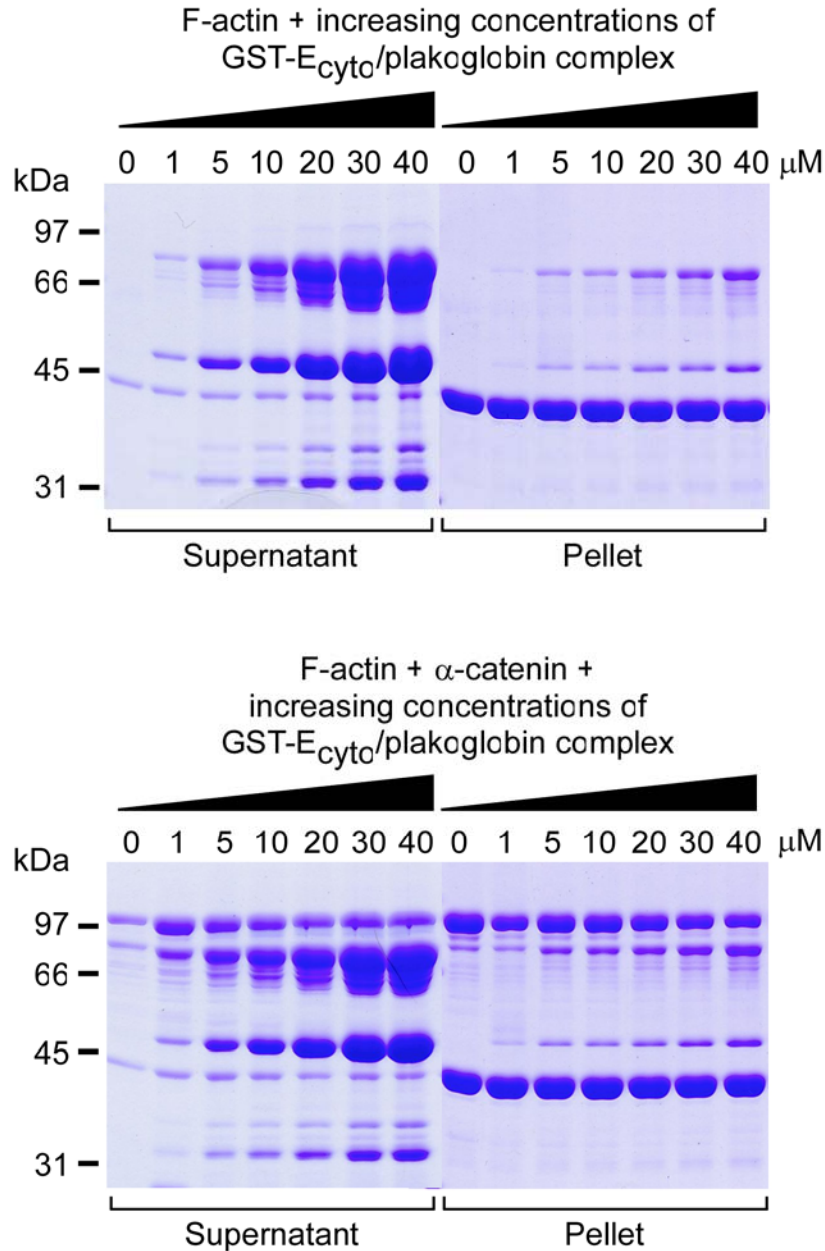


Figure S1. F-Actin Pelleting Assay with GST-E-Cadherin-Plakoglobin- α -Catenin Complex

F-actin was incubated without or with 5 μ M α -catenin and increasing amounts of GST-E-cadherin-plakoglobin complex. Supernatant and pellet of each concentration point were analyzed by SDS-PAGE. Note that a degradation product of α -catenin, which can be seen in the supernatant of 0 concentration point with α -catenin runs at the same molecular weight as plakoglobin.